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Anticardiolipin Antibodies in Acute Myeloid Leukemia: Prevalence and Clinical Significance

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This prospective study was designed to explore the prevalence and the clinical and prognostic significance of anticardiolipin (ACL) antibodies in patients with acute myeloid leukemia (AML). The study includes 37 consecutive AML patients >15 years old without previous history of thromboembolism, recurrent fetal loss, or autoimmune disease and with no evidence of infection at the time of enrollment. ACL antibodies were found in 25 patients (68%). None of the patients had high positive titers; 8 had moderately positive while 17 had low positive ACL antibody titers. ACL antibody positivity did not predict response to chemotherapy and was not correlated with age, gender, FAB class, platelet and white blood cell counts at presentation, and the risk of thromboembolism. ACL antibody titers did correlate, however, with AML activity in the majority of patients (93%) during 4–19 months of follow up. These results demonstrate a high prevalence of ACL antibodies in AML patients and suggest that serum ACL antibodies may be a useful adjunct in predicting relapse and documenting disease activity and therapy response. Am. J. Hematol. 57:139–143, 1998. © 1998 Wiley-Liss, Inc.

Key words: anticardiolipin antibodies; acute myeloid leukemia; lupus anticoagulant

INTRODUCTION

Antiphospholipid (APL) antibodies are a heterogeneous family of autoimmune and alloimmune immunoglobulins, directed predominantly against protein-phospholipid complexes [1,2]. APL antibodies include lupus anticoagulant (LA), which prolongs phospholipid dependent coagulation in vitro and anticardiolipin (ACL) antibodies detected by solid phase immunoassay. Because in some patients there is a lack of concordance between the results of these tests, most investigators now agree that LA and ACL antibodies define separate subgroups of antibodies [2–4]. APL antibodies have been detected in patients with systemic lupus erythematosus and other autoimmune diseases, infections, drug-induced conditions, and in approximately 10% of the healthy population without predisposing factors [5,6]. ACL antibodies have been found in 22% of patients with solid malignancies who also suffered from an increased risk of malignancy associated thrombosis [7]. Only sporadic reports of the association of APL antibodies (e.g., LA or ACL) with hematological malignancies such as non-Hodgkin's lymphoma [8], plasma cell dyscrasia [9], hairy cell leukemia [10], and acute lymphoblastic leukemia [11] were published. Also a single investigation describing prevalence of 26% of APL antibodies in a small series of patients with acute myeloid leukemia (AML) was reported [12]. The purpose of this prospective study was to determine the prevalence and the clinical and prognostic significance of ACL antibodies in patients with AML.

MATERIALS AND METHODS

Patients

The study population consisted of consecutive patients with AML hospitalized in the Hematology Department, Hadassah University Hospital, Jerusalem, between June 1995 and October 1996; follow-up continued through March 1996. Patients were eligible for this study if they suffered from untreated, de novo or secondary, AML, or relapsed AML following at least 6 months of unmaintained remission. Diagnosis of AML was established according to the French-American-British (FAB) criteria after examination of hematoxylin-eosin stained smears of

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peripheral blood and bone marrow and cytochemical studies. Immunophenotypic flow cytometry and cytogenetic studies were also performed. Patients were excluded from the study if they had a previous history of thromboembolism, recurrent fetal loss, or an autoimmune disease, if their age was less than 15 years; if they had been treated with immunosuppressive agents, immuno- or chemotherapy within the previous 6 months or if they had clinical, microbiologic, or roentgenographic evidence of infection at the time of the ACL antibody test.

Patients up to the age of 65 years were treated by an induction regimen consisting of continuous infusion of cytarabine 100 mg/m^2/day for 7 days and daunorubicin 45 mg/m^2/day for 3 days. If remission was achieved, two consolidation courses consisting of high-dose cytarabine (3 g/m^2 twice daily for 6 days) and a course of mitoxantrone 12 mg/m^2/day for 3 days with etoposide 100 mg/m^2/day for 5 days were administered. Patients aged 51–65 years were treated with a reduced dose of cytarabine (2 g/m^2). Patients with acute promyelocytic leukemia were treated with all-trans retinoic acid 45 mg/m^2 until remission induction, before institution of the above regimen. Patients who failed to achieve complete remission (CR) or patients with relapsing AML were treated with a salvage protocol consisting of cytarabine 3 g/m^2/day for 5 days and mitoxantrone 20 mg/m^2/day for 2 days. Eligible patients with poor prognostic factors or following disease relapse were referred for bone marrow transplantation (BMT). The therapeutic regimen in patients aged 66–77 years varied according to their general health condition.

Antibody Assay

Laboratory evaluation of serum ACL antibody titers was performed at diagnosis, before every chemotherapy course, at 3-month intervals following completion of chemotherapy, and at relapse. Ig G and Ig M ACL antibodies were measured in serum stored at −20°C by an enzyme linked immunosorbent assay (ELISA), using a commercially available kit (Selisa™, Cambridge Life Sciences plc, Cambridgeshire, England). The assay was calibrated against an international standard, one unit being defined as the cardiolipin-binding activity of 1 μg of affinity-purified IgG/IgM-ACL antibody [13]. All ACL antibody determinations were performed blindly at two different runs, including other unknown sera. The normal levels were defined by measuring IgG- and IgM-ACL antibody titers in controls. Results were considered positive when the values exceeded the mean controls by 3 standard deviations (>7.5 u/ml for both IgG- and IgM-ACL antibodies). Titers between 7.5 and 20 u/ml were considered low positive, between 20 and 80 u/ml moderate positive, and over 80 u/ml high positive. Antinuclear antibodies (ANAs) were determined by indirect immunofluorescence on Hep-2 cell slides.

TABLE I. Characteristics of Anticardiolopin (ACL) Antibody Positive and Negative Patients With Acute Myeloid Leukemia

Characteristic	ACA-positive (25 patients)	ACA-negative (12 patients)
Age		
Range	15–77	19–71
Mean	44	47
Sex		
Male	12	8
Female	13	4
FAB		
M–0	2	–
M–1	5	2
M–2	5	1
M–3	1	1
M–4	2	3
M–5	5	1
Secondary	5	4
Thrombosis	1	1
Chemoresistance	4	1

Statistical Analysis

The differences between the ACL antibody-positive and ACL antibody-negative patients were evaluated using the chi-square or Fisher's exact tests when applicable. A *P* value of less than 0.05 was defined as statistically significant.

RESULTS

Thirty-seven AML patients were eligible for this study. Their clinical characteristics are presented in Table I. ACL antibodies were found in the serum of 25 patients (68%). They were of IgG, IgM, or both types in 11, 6, and 8 patients, respectively (Table II). None of the patients had high positive ACL titer. Moderately positive IgG- and IgM-ACL antibody titers were detected in 3 and 5 patients, respectively, while the majority of patients had low positive titers. ANAs were found in a single patient with ACL antibodies. As shown in Table I, no correlation was found between the presence of ACL antibodies and the following parameters: age, gender, FAB class, chemoresistance, platelet and white blood cell count at presentation, and the length of remission (data not shown). Thrombotic phenomena (cerebrovascular accidents) were observed in one ACL-positive and one ACL-negative patient.

Fifteen ACL antibody positive patients were followed for 4 to 19 months (Fig. 1). Ten patients had chemotherapy-responsive leukemia and had achieved remission lasting for at least 3 months (Fig. 1R), while 5 patients had chemotherapy-resistant (4 patients) or rapidly relapsing (1 patient) AML (Fig. 1U). The number of follow-up ACL antibody measurements in Figure 1 is 19, since 4 patients (two in Fig. 1R and two in Fig. 1U) had both IgG- and IgM-ACL antibodies and are shown in both

TABLE II. Serum Anticardiolipin (ACL) Antibody Concentrations in Acute Myeloid Leukemia Patients

ACL antibodies	Number of patients	Antibody titer	
		Mean ± SD	Range
IgG	11	12.6 ± 5.1	8.0 – 25.0
IgM	6	27.3 ± 16.2	8.0 – 45.0
IgG and IgM	8		
IgG		15.9 ± 4.2	9.0 – 21.5
IgM		19.0 ± 13.7	9.0 – 54.0

curves. In 5 patients with chemoresistant or rapidly relapsing leukemia (within 3 months of therapy termination), ACL antibodies were persistently positive (Figs. 1U, 2A). One of these patients with chemoresistant-leukemia had both IgG- and IgM-ACL antibodies at diagnosis. During chemotherapy IgM-ACL antibodies disappeared, while IgG-ACL antibodies persisted.

Ten patients achieved remission, lasting at least 3 months following completion of chemotherapy. In eight patients ACL antibody titers fell below 7.5 u/ml following achievement of morphologic and cytogenetic CR (Figs. 1R 2B, C). Five of those patients suffered from AML relapse, accompanied by reappearance of ACL antibodies in four (Fig. 2B, representative case). Three patients have remained in CR for 4 to 13 months with persistently negative ACL antibodies (Fig. 2C, representative case). An additional two patients, in chemotherapy-maintained second CR following initial relapse, had persistently positive ACL antibodies for 8 and 10 months, respectively (Fig. 1R). One of these patients had AML relapse, while the other is still in maintained second CR.

Among the patients with baseline negative ACA antibodies, one patient with secondary AML refused chemotherapy. During the follow-up she became persistently ACL-positive. In four patients, a transient ACL positivity coincidental with infection was observed.

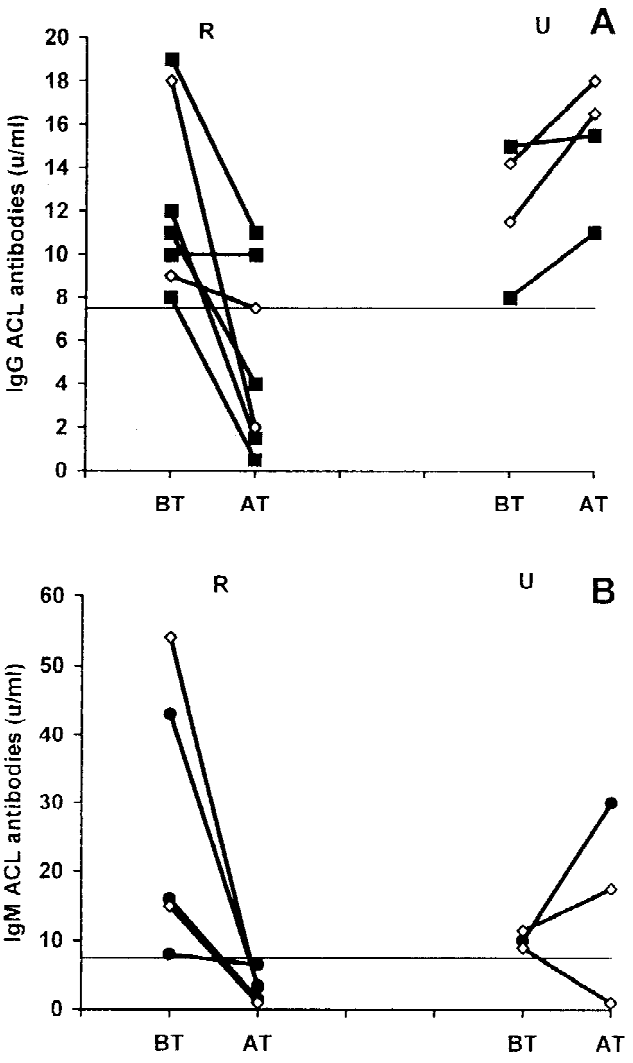

Fig. 1. Serum IgG (A) and IgM (B) anticardiolipin (ACL) antibody titers at diagnosis, before treatment of acute myeloid leukemia (BT) and following chemotherapy (AT), in 5 patients with either chemoresistant or rapidly relapsing leukaemia (U) and in 10 chemotherapy responding patients (R). Four patients had both IgG- and IgM-ACL antibodies. Patients with both IgG- and IgM-ACL antibodies are presented by open rhombi, patients with IgM-ACL antibodies by closed circles, and patients with IgG-ACL antibodies by closed squares.

DISCUSSION

Increased incidence of certain autoantibodies was documented in patients with malignancy [14]. For example, in patients with carcinoma of breast, the presence of autoantibodies is associated with a less favorable prognosis [15]. There is also growing evidence on the association of APL antibodies (e.g., LA and ACL antibodies) with malignancy [7,8]. These antibodies, directed predominantly against complexes of negatively charged phospholipids with protein, are commonly found in autoimmune disorders and are characteristic of the primary anti-phospholipid syndrome [5,16]. It has to be emphasized, that APL antibody population is very heterogeneous (e.g., LA, ACL, β_2 glycoprotein 1 [β_2GPI] antibodies), resulting in a lack of concordance among the results of different laboratory methods even in the same patient [2–4]. Therefore, patients with LA antibodies who are ACL antibodies negative and vice versa, may be identified.

A 22% prevalence of ACL antibodies was described in malignancy with an increased risk of thromboembolism [7], but ACL antibodies were uncommonly reported in hematological malignancies. To the best of our knowledge, there is a single study demonstrating LA and ACL antibody prevalence of 26% in a small group of 19 patients with de novo AML [12].

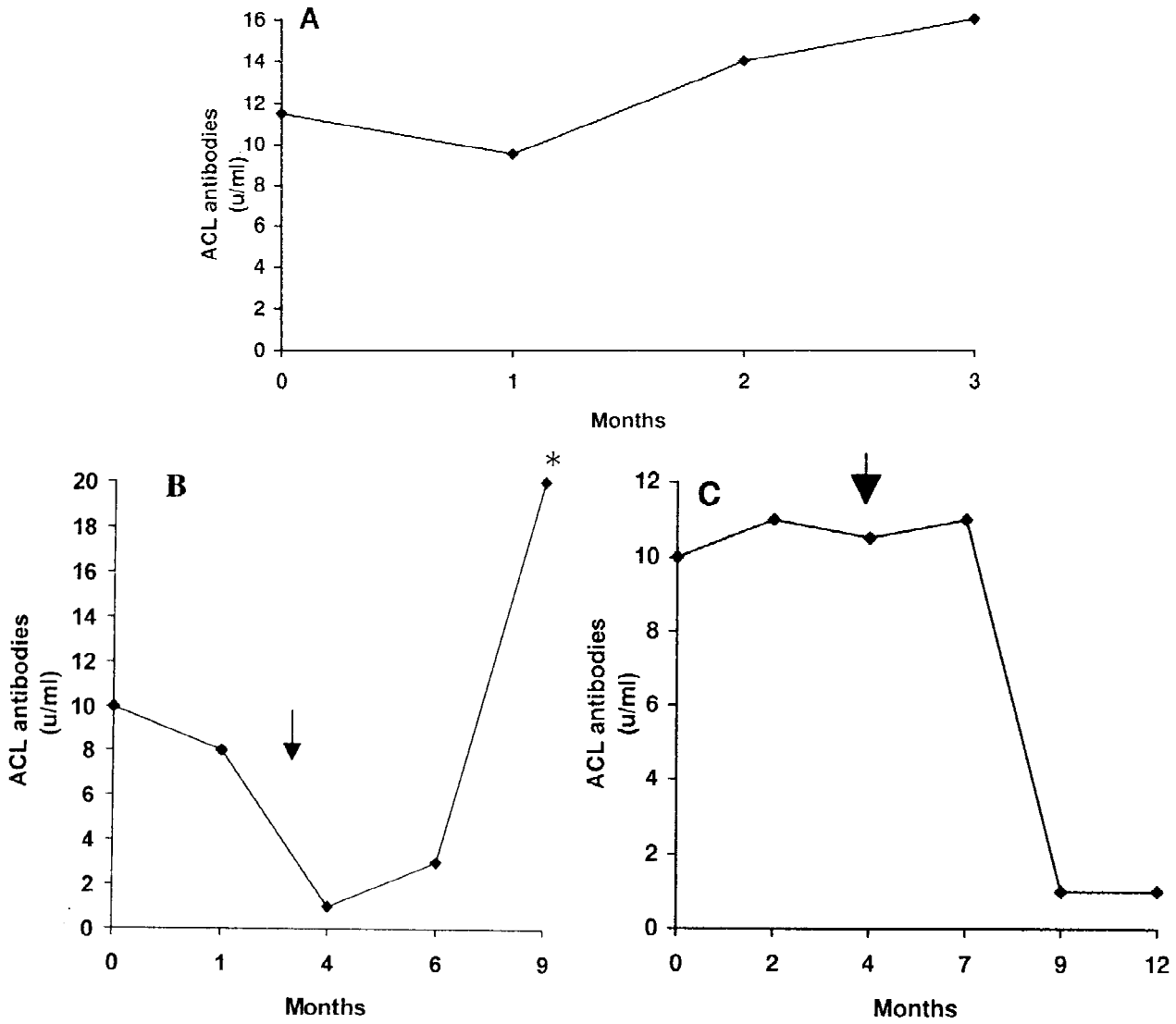

Fig. 2. Follow-up of serum anticardiolipin (ACL) antibody levels in representative cases receiving chemotherapy for acute myeloid leukemia (AML). A: Patient with chemoresistant AML. B: Patient who achieved a complete remission (arrow) following chemotherapy, but subsequently relapsed (*). C: Patient who achieved a long-standing complete remission (arrow) following chemotherapy.

To further elucidate the possible association between ACL antibodies and AML and its clinical significance, we performed this prospective study in a larger group of AML patients. Our study demonstrates a 68% prevalence of ACL antibodies in this population. This prevalence markedly exceeds the reported 10% prevalence in the healthy population [6]. Patients with disorders known to be associated with ACL antibodies, such as autoimmune diseases, infections, thromboembolism, and recurrent fetal loss, were excluded from the study, thus suggesting that ACL antibodies in our patients are related to the basic disease, AML. Furthermore, the persistence of ACL antibodies in patients with resistant disease and early relapse, its disappearance following achievement of CR and resurgence following relapse may suggest a non-coincidental association between ACL antibodies and AML.

A similar pattern of ACL antibody response to treatment of underlying malignancy was reported in patients with non-Hodgkin's lymphoma and solid tumors [7,8]. However, the etiopathogenetic relationship between AML and ACL antibodies is not clear. Several mechanisms may be suggested for ACL antibodies production: (1) a change in the membrane of leukemic cells may result in exposure of protein-phospholipid complexes leading to immune system response. Plasma membrane alterations have been reported in leukemic patients, especially in chronic myeloid leukemia [17,18]; (2) leukemic cells may secrete protein-phospholipid complexes or its components with subsequent complex assembly in plasma,

initiating an immune response; (3) production of monoclonal immunoglobulins with LA or ACL activities by malignant cells. Although such immunoglobulins have been reported in patients with lymphoma [8] and plasma cell dyscrasia [9,19], this mechanism is unlikely to apply to a non-B cell malignancy like AML.

The structure of the protein-phospholipid antigen complexes to which ACL antibodies are directed in AML patients is unclear. Whether ACL activity in these patients is dependent on the presence of prothrombin, β_2GPI, or other proteins is unclear. Several studies had demonstrated that the β_2GPI-dependent binding to phospholipids can be used to discriminate between autoimmune ACL antibodies and those found in patients following infections [20]. The presence of β_2GPI in AML patients was not yet tested. Further studies are needed to elucidate the mechanisms and the antigenic nature of ACL antibodies in AML patients.

The association between thrombosis and ACL antibodies is well established in patients with primary antiphospholipid syndrome and autoimmune diseases [5]. Recently, an increased risk of thromboembolism had been observed in ACL-positive patients with solid malignancies [7]. In our and Stasi et al.'s [12] AML patients, the presence of ACL antibodies was not associated with an increased risk of thromboembolism. This may result from either low ACL antibody titers or due to thrombocytopenia, frequently found in AML patients. The relatively small study population size can also be an explanation, since thromboembolism is a rare phenomenon in the AML population.

Our study suggests that the repeated measurement of serum ACL antibodies may be a useful adjunct in documenting disease activity and response to therapy in AML patients. Disappearance of ACL antibodies accompanied achievement of CR, while its resurgence predicted AML relapse. ACL antibody persistence was observed in patients having early relapse or suffering from resistant leukemia. In addition, ACL antibodies persisted in two patients during second CR, a situation with a high relapse rate. It has been suggested that sustained antitumor immune response may be due to ''dormant'' tumor cells that provide continued antigenic stimulation [21].

Persistence of ACL antibodies during CR in patients with early relapse or in second CR may be a result of such low-grade antigenic stimulation due to a minute number of tumor cells that still have potential for relapse but are morphologically undetectable.

In conclusion, our findings demonstrate a high prevalence of ACL antibodies in the serum of AML patients. ACL antibodies were not correlated with particular clinical features, but might be a reliable marker of disease activity. Further studies clarifying the etiopathogenetic relation between AML and ACL antibodies are needed.

REFERENCES

1. Arnout J: The pathogenesis of the antiphospholipid syndrome: A hypothesis based on parallelisms with heparin-induced thrombocytopenia. Thromb Haemost 75:536, 1996.
2. Harris EN: Antiphospholipid antibodies. Br J Haematol 74:1, 1990.
3. Derksen RHWM, Hasselaar P, Blokzijl L, Meyling FHJG, DeGroot PG: Coagulation screen is more specific than the anti-cardiolipin antibody ELISA in defining a thrombotic subset of lupus patients. Ann Rheum Dis 47:364, 1988.
4. Sarice M, Cicella A, Griggi T, Garofalo T, Nicodemo G, Pittoni V, Pontieri GM, Lenti L, Valesini G: Anticardiolipin and anti-β_2-GPI are two distinct populations of autoantibodies. Thromb Haemost 75:303, 1996.
5. Love PE, Santoro SA: Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Ann Intern Med 112:682, 1990.
6. Shi W, Krilis SA, Chong BH, Gordon S, Chesterman CN: Prevalence of lupus anticoagulant and anticardiolipin antibodies in a healthy population. Aust NZ J Med 20:231, 1990.
7. Zuckerman E, Toubi E, Dov Golan T, Rosenvald-Zuckerman T, Sabo E, Shmuel Z, Yeshurun D: Increased thromboembolic incidence in anti-cardiolipin-positive patients with malignancy. Br J Cancer 72: 447, 1995.
8. Ciaudo M, Horellou MH, Audouin J, De Carbonnieres C, Conard J, Samama M: Lupus anticoagulant associated with primary malignant lymphoplasmacytic lymphoma of the spleen. Am J Hematol 38:271, 1991.
9. Bellotti V, Gamba G, Merlini G, Montani N, Bucciarelli E, Stoppini M, Ascari E: Study of three patients with monoclonal gammopathies and lupus-like anticoagulants. Br J Haematol 73:221, 1989.
10. Duncombe AS, Dalton RG, Savidge GF: Lupus-type coagulation inhibitor in hairy cell leukemia and resolution with splenectomy. Br J Haematol 65:120, 1987.
11. Donner M, Bekassy NA, Garwicz S, Holmberg L, Wiebe T: Cerebral infarction in a girl who developed anticardiolipin syndrome after acute lymphoblastic leukemia. Pediatr Hematol Oncol 9:377, 1992.
12. Stasi R, Stipa E, Masi M, Oliva F, Sciarra A, Perroti A, Zaccari G, Papa G: Antiphospholipid antibodies: prevalence, clinical significance and correlation to cytokine levels in acute myeloid leukemia and non-Hodgkin's lymphoma. Thromb Haemost 70:568, 1993.
13. Harris EN, Gharavi AE, Patel SP, Hughes GRV: Evaluation of the anti-cardiolipin antibody test: Report of an international workshop held 4 April 1986. Clin Exp Immunol 68:215, 1987.
14. Tannenberg AEG, Muller, HK, Cauchi MN, Nairin RC: Incidence of autoantibodies in cancer patients. Clin Exp Immunol 15:153, 1973.
15. Wasserman J, Glas U, Blomgren H: Autoantibodies in patients with carcinoma of breast. Clin Exp Immunol 19:417, 1975.
16. Devine DV, Brigden ML: The antiphospholipid syndrome. Postgrad Med 99:105, 1996.
17. Ackerman GA: Ultrastructural histochemical alteration of the plasma membrane in chronic myelocytic leukemia. Blood 63:869, 1975.
18. Baker MA, Taub RN, Whelton CH, Hindenburg A: Aberrant sialylation of granulocyte membranes in chronic myelogenous leukemia. Blood 63:1194, 1984.
19. Gleicher M, Friberg, J: IgM gammopathy and the lupus anticoagulant. JAMA 253:3278, 1985.
20. McNally T, Purdy G, Mackie IJ, Machin SJ, Isenberg DA: The use of an anti-β_2-glycoprotein-I assay for discrimination between anticardiolipin antibodies associated with infection and increased risk of thrombosis. Br J Haematol 91:471, 1995.
21. Khazaie K, Prifi S, Beckhove P, Griesbach A, Russell S, Collins M, Schirmacher V: Persistence of dormant tumor cells in the bone marrow of tumor cell vaccinated mice correlates with long term immunologic protection. Proc Natl Acad Sci USA 91:7430, 1994.